Detection of vancomycin resistant enterococci (VRE)

Although clinical laboratories can reliably detect high level resistance to vancomycin, there are reports of poor proficiency in the detection of low-level inducible resistance to vancomycin.

Pendle et al (8) found that the Vitek 2 correctly identified only 2 of 16 isolates of E. faecium vancomycin-resistant isolates (VRE) with low-level vancomycin resistance. The remaining were not identified or identified as aerococcus viridans. Disk testing using the CLSI method, was unreliable for detection of vancomycin resistance upon primary isolation.

Heteroresistance of E. faecium to vancomycin, was reported by Alam et al (1) in 2001. The heterogeinicity of the VRE isolate was observed only by Etest (colonies inside the zone), automated Microscan or microdilution were not adequate for detecting heteroresistance.
In some European countries up to 5 % of non-hospitalised persons could be asymptomatically colonised with VRE (2). Hospital outbreaks of VRE are almost exclusively caused by a specific genogroup of vancomycin-resistant E. faecium characterised by co-resistance to ampicillin (and ciprofloxacin) and the presence of the variant esp gene (3, 7).

VanB phenotype and vanA genotype Enterococcus faecium with heterogeneous expression of glycopeptide resistance are reported form Korea (5) and China (14).
Outbreaks of vancomycin resistant E. faecium expressing VanD-like phenotype associated with a vanA genotype are reported from France (4) and Korea (9). Interpretation of MICs for vancomycin was difficult since most of the isolates presented heterogeneous expression of resistance, with colony growth in the E-test zone. Was better visible after 48 hours incubation. Colonies growing inside the zone of inhibition once retested, showed a homogeneous phenotype of resistance to vancomycin. Teicoplanin was not effective against vanD-vanA VRE strains both in vitro and in vivo.

Neves (11) in Brazil reports the emergence of vanA genotype among E. gallinarum from patients in a University Hospital in Rio de Janeiro. Resistance to teicoplanin, when testing motile enterococci, may be a clue to the presence of the vanA gene.

Dobbs et al (6) report the nosocomial spread of Enterococcus faecium resistant to both vancomycin and linezolid in a tertiary care Medical Center. Exposure to fluoroquinolones may be important for the emergence of linezolid resistance.

Souli et al (13) in a prevalence survey of faecal carriage in a tertiary care university hospital in Athens (Greece) found 6 isolates of vancomycin and linezolid resistant vanA positive E. faecium.

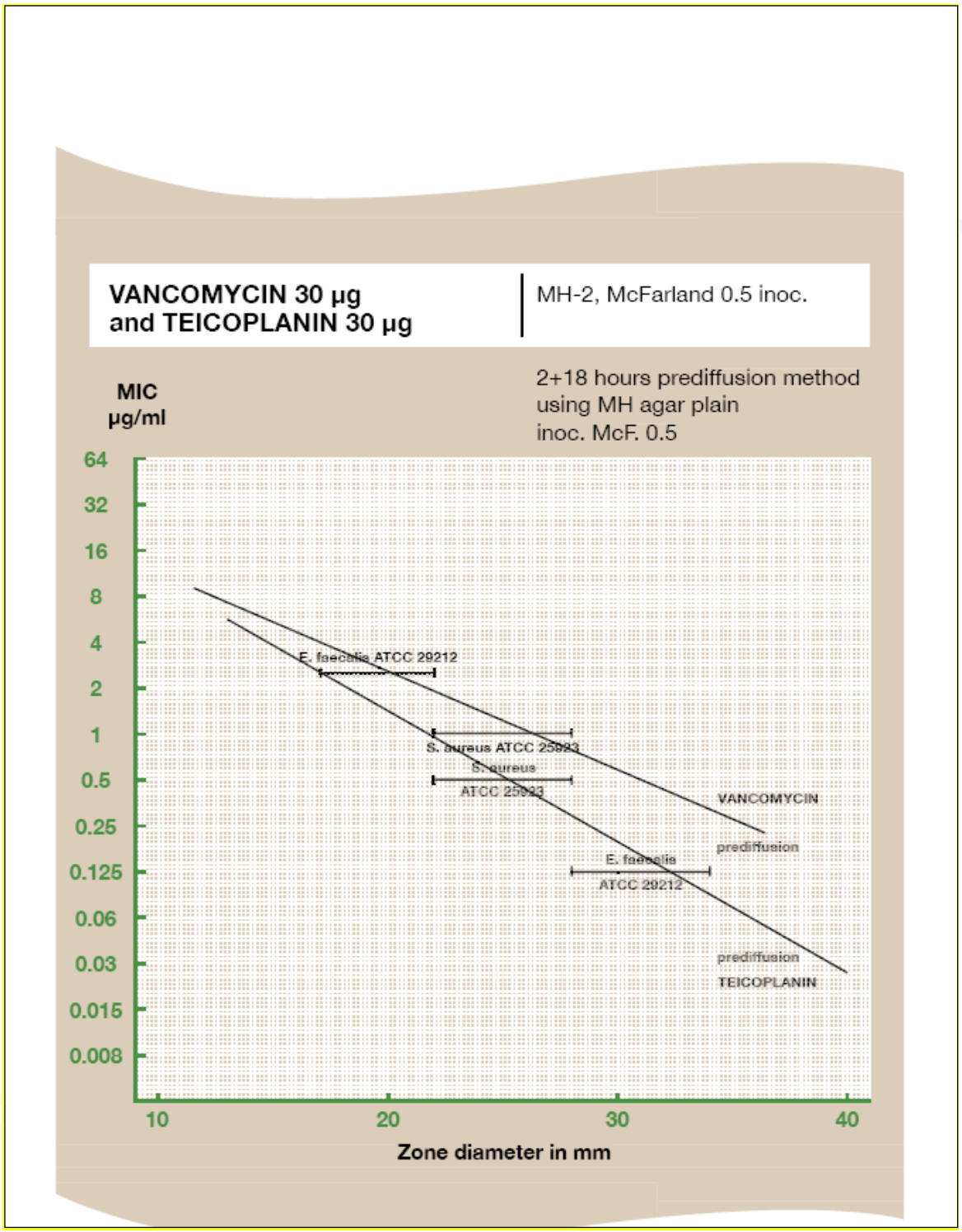
Procedure

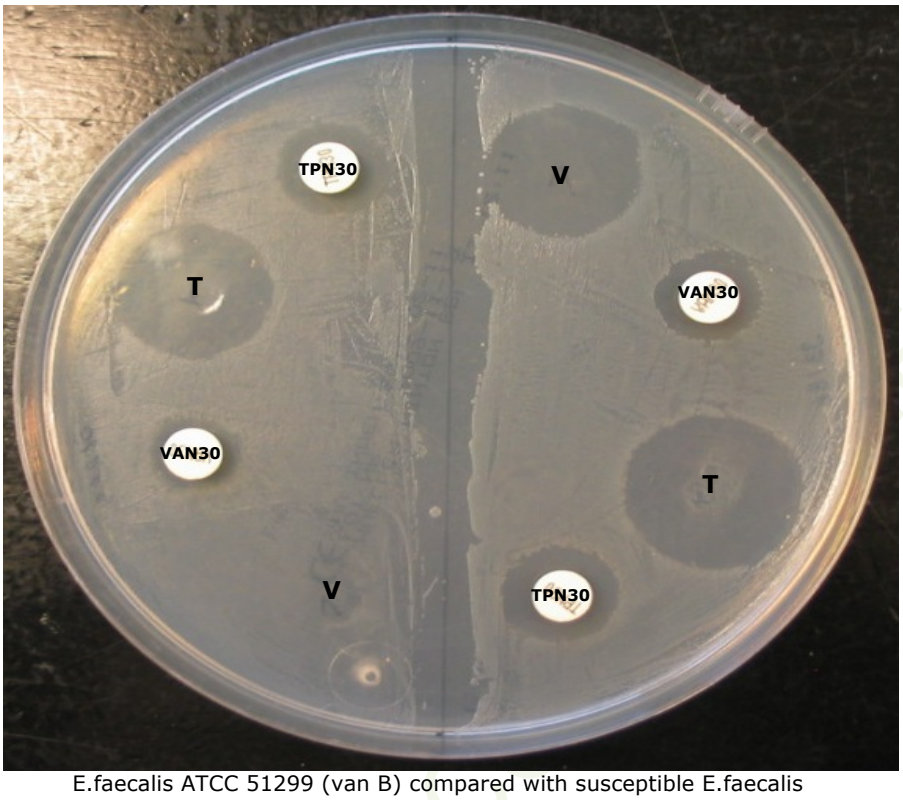
1) One Teicoplanin 30 ug and one Vancomycin 30 ug Neo-sensitabs are placed on an uninoculated plate containing plain MH agar.
2) After 2 hours at room temperature, the tablets are removed (knocking the plate against the table) and the short names (VAN and TEI) are written on the back of the plate for identification purposes.
3) The plate is maintained at room temperature for further 18-22 hours (overnight)
4) The plate is now inoculated with the strain to be tested using McFarland 0,5 inoculum and additional antimicrobial disks (Neo-sensitabs) may be added using a dispenser. Thereafter, incubate at 35 degrees overnight.
5) The zones of inhibition are measured and compared with the corresponding zone breakpoints.

Interpretation

VanA:	Vancomycin R and Teicoplanin R: no zone of inhibition
Van B:	Vancomycin zone < 16 mm (hazy edge) and Teicoplanin zone >20 mm.
VanC:	Vancomycin zone < 12 mm (sharp edge). Teicoplanin zone >20 mm.

VanB Phenotype and vanA genotype: Vancomycin no zone, Teicoplanin zone < 6 mm (R)
VanC Phenotype and vanA genotype: Vancomycin no zone, Teicoplanin zone <12 mm (R)
VanD Phenotype and vanA genotype: Vancomycin no zone, Teicoplanin zone < 16mm (R)


VANCOMYCIN 30 µg
and TEICOPLANIN 30 µg
MH-2, McFarland 0.5 inoc.
2+18 hours prediffusion method
using MH agar plain
inoc. McF. 0.5
MIC
µg/ml
64
32
16
8
4
2
1
0.5
0.25
0.125
0.06
0.03
0.015
0.008
E. faecalis ATCC 29212
S. aureus ATCC 25923
S. aureus
ATCC 25923
VANCOMYCIN
prediffusion
E. faecalis
ATCC 29212
prediffusion
TEICOPLANIN
10
20
30
40
Zone diameter in mm



E.faecalis ATCC 51299 (van B) compared with susceptible E.faecalis

V=Vancomycin, T=Teicoplanin

References

1) Alam MR et al: Heteroresistance to vancomycin in Enterococcus faecium. J Clin Microbiol.**39**,3379-3381,2001.
2) Mascini EM et al: Vancomycin-resistant enterococci: consequences for therapy and infection control. Clin Microbiol Infect **11**,suppl 4, 43-56,2005.
3) Willems RJL et al: Global spread of vancomycin-resistant E. faecium from distinct nosocomial genetic complex. Emerg Infect Dis **11**,821-827,2005.
4) Naas T et al: First nosocomial outbreak of vancomycin-resistant E. faecium expressing a VanD-like phenotype associated with a vanA genotype. J. Clin Microbiol,**43**,3642-49,2005.
5) Jae-Hoon Song: High frequency of vancomycin-resistant E. faecium isolates with VanB phenotype and vanA genotype in Korean hospitals. Diagn Microbiol Infect Dis, **56,**401-406,2006.
6) Dobbs TE et al Nosocomial spread of E. faecium resistant to vancomycin and linezolid in a terciary care medical center. J. Clin Microbiol **44**,3368-3370,2006.
7) Werner G et al: Emergence and spread of vancomycin resistance among enterococci in Europe. Eurosurveillance,**13**,1-16 November 2008.
8) Pendle S et al.: Difficulties in detection and identification of E. faecium with low-level inducible resistance to vancomycin, during a hospital outbreak. Clin Microbiol Infect **14**,853-857,2008.
9) Jae-Hoon Song et al: Clinical implications of vancomycin-resistant E. faecium (VRE) with VanD phenotype and vanA genotype. J. Antimicr Chemother. **61**,838-844,2008.
10) Khan SA et al: Heteroresistance to vancomycin and novel point mutations in Tn1546 of E. faecium ATCC 51559.Int J Antimicr Agents **31**,27-36,2008.
11) Neves FPG et al: Emergence of the vanA genotype among E. gallinarum isolates colonising the intestinal tract of patients in a university hospital in Rio de Janeiro, Brazil. Int J Antimicr Agents **33**,211-215,2009.

12) San Millan A et al: Van-B type E. faecium clinical isolate succesively inducibly resistant to, dependent on, and constitutively resistant to vancomycin. Antimicr Ag Chemother. **53**,1974-1982,2009.
13) Souli M et al: Colonisation with vancomycin and linezolid-resistant E. faecium in a university hospital: molecular epidemiology and risk factor analysis. Int J Antimicr Agents **33**,137-142,2009.
14) Li Gu et al: A new Tn1546 type vanB phenotype-vanA genotype vancomycin-resistant E. faecium isolates in mainland China. Diagn Microbiol Infect Dis,**63**,70-75,2009.